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Plant stages with biotic, indirect defences are more palatable and suffer less herbivory than their undefended counterparts

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Plants have evolved several anti-herbivory strategies, including direct defences, such as mechanical and chemical defences, and indirect or biotic defences, such as the recruitment of defending animals. We examined whether the investment plants make in direct defences differs between those which do and do not invest in biotic defences, by comparing standing herbivory and palatability of congeneric species with and without indirect defences at two ontogenetic stages: before and after the onset of indirect defences. We used *Cordia alliodora* and *Croton suberosus* as the species with indirect defences and *Cordia elaeagnoides* and *Croton pseudoniveus* as the species without indirect defences. We predicted that herbivores would prefer to eat species and stages with indirect defences to those without them. As predicted, we found that herbivores preferred species and ontogenetic stages with indirect defences in all cases. Overall, however, natural levels of herbivory were lower in species with indirect defences. We conclude that indirect defences offer effective protection against herbivores and posit that their recruitment allows plants to reduce investment in other defence mechanisms. Our results support the notion that plants trade-off between direct and indirect defensive strategies.

ADDITIONAL KEYWORDS: anti-herbivore defences – herbivore preference – Mexico – mutualistic defenders – plant ontogeny – trade-offs – tropical dry forest.

INTRODUCTION

Plants protect themselves against herbivores with a diverse array of mechanisms, including direct and indirect defences. Direct defences are traits that decrease plant quality as food for herbivores, thereby reducing herbivore damage (Gowda *et al*., 2003; Rohde, Molis & Wahl, 2004). Indirect or biotic defences result from traits that attract animals, which in turn defend the plant from its herbivores (Janzen, 1966, 1969; Schemske, 1980; Domínguez, Dirzo & Bullock, 1989; Di Giusto *et al*., 2001; Cuautle & Rico-Gray, 2003; Del Val & Dirzo, 2004; Kost & Heil, 2005). Anti-herbivore defences often differ among ontogenetic stages within plant species and there are two primary hypotheses to explain patterns in this variation. The first explanation is concerned with resource allocation and proposes that young plants are unable to produce high levels of defences because they lack the resources to produce them (Herms & Mattson, 1992). The second explanation, the optimal-defence hypothesis, suggests that younger stages are more vulnerable to herbivore attacks and should therefore be better defended (Bryant *et al*., 1992). Combining these two explanations, Boege & Marquis (2005) predicted a decrease in the levels of defences from seedlings to the juvenile stage and an increase in defences from the juvenile to the mature stage. Some of the patterns predicted by Boege & Marquis (2005) have been supported by a meta-analysis of available studies, but the broader picture is more complex: the relationship between plant age and defence level differs between plant life

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histories, herbivore groups and geographical location (Barton & Koricheva, 2010). In particular, for non-boreal woody plants, Barton & Koricheva (2010) detected a general increase in the level of defences from the seedling to the juvenile to the mature stages. From their results, Barton & Koricheva (2010) concluded that mechanistic constraints probably determine the defence strategy adopted by each species.

Mechanistic constraints such as resource limitation and physiological trade-offs influence ontogenetic variation in plant defences in many systems. For instance, certain secondary compounds are stored in specialized tissue that is not present in young seedlings, leading to an ontogenetic increase in the use of these defences when the tissues become available (Goralka & Langenheim, 1996; Goralka, Schumaker & Langenheim, 1996 – cited in Barton & Koricheva, 2010). Likewise, some indirect defences rely on tissues and/or structures that are not present in young seedlings. This led Boege & Marquis (2005) to predict that, unlike direct defences, these indirect defences will increase as plants grow from seedlings to juveniles.

Some plants, known as myrmecophytes (Webber *et al*., 2007), produce feeding rewards for ants (nectar or nutritious bodies). Frequently, mymecophytes produce specialized structures called domatia, in which ants nest. Domatia may also, in some cases, provide food for the ants (Fiala & Maschwitz, 1992; Gaume, McKey & Terrin, 1998). In turn, ants protect host plants from herbivore attack (Janzen, 1966; Schupp, 1986; Madden & Young, 1992; Rocha & Bergallo, 1992; Fiala *et al*., 1994; Chamberlain & Holland, 2009; Rosumek *et al*., 2009). Other plants produce unconcealed nectar, in floral or extrafloral nectaries, to attract defending wasps (Domínguez *et al*., 1989; Cuautle & Rico-Gray, 2003). Wasps visit the plant to feed on the nectar and, whilst doing so, remove any herbivores that they encounter (Domínguez *et al*., 1989).

In all known cases, plants that rely on these mutualistic relationships do not begin their lives with the necessary indirect defences to acquire or maintain them. Instead, they must do so during their ontogeny (Del Val & Dirzo, 2003). The absence of indirect defences during the early stages of plant growth has led to the suggestion that young (uncolonized) plants may be more reliant upon direct defences than older (colonized) ones (Collantes, Gianoli & Niemeyer, 1997; Heil & McKey, 2003).

Studies investigating the anti-herbivore defences of uncolonized myrmecophytes have varied widely in their support of the hypothesis that uncolonized young plants rely more upon direct defences than colonized ones. Nomura, Itioka & Murase (2001) showed that, in captivity, generalist herbivores ate more and survived better on leaves clipped from plants after the initiation of symbiosis with ants than from plants before the initiation of this symbiosis in three *Macaranga* tree species. In contrast, Trager & Bruna (2006) showed that the specialist beetle *Coptocycla leprosa* fed equally on leaves from 1- and 5-year-old individuals of *Cordia alliodora* during laboratory trials (see also Del Val & Dirzo, 2003).

Furthermore, when comparing investment in direct defences between congeneric species with and without indirect defences, different studies have reached different conclusions. While some studies found significant differences in the amount of direct defences used by congeneric species with and without indirect defences (e.g. Rehr, Feeny & Janzen, 1973; Heil, Staehelin & McKey, 2000; Eck *et al*., 2001), others have found no such differences (Steward & Keeler, 1988; Heil *et al*., 2002; Rudgers, Strauss & Wendel, 2004). Clearly, the lack of congruence among studies highlights a need for studies that comprehensively control for several confounding factors.

Given the discordant results of past studies, we wanted to examine if two indicators of plant investment in direct defences, herbivory and palatability (see Del Val & Dirzo, 2003), differ between plants with and without indirect defences. For this purpose, we compared the standing herbivory and palatability of two congeneric species with and without indirect defences at two ontogenetic stages: before and after the onset of indirect defences. We predicted that herbivores would prefer to feed on species and stages with indirect or biotic defences.

MATERIAL AND METHODS

Study site

We conducted this study at the Chamela Biological Station, within the Chamela–Cuixmala Biosphere Reserve (19°30′N, 105°03′W, Jalisco, Mexico), where the predominant vegetation is seasonally dry tropical forest. The area is characterized by its extreme seasonality. Average annual temperature is 25 °C and average annual precipitation is 750 mm, with a marked dry season from November to June (Bullock, 1986; Stoner, 2005).

The study was conducted during the rainy season of 2006 and field observations were carried out during the beginning of August, approximately 1 month after main leaf expansion occurred (Boege, 2004).

Study species

Cordia alliodora and *Cordia elaeagnoides* (Boraginaceae) are widespread in the Neotropical Region. *Cordia alliodora* presents traits of a myrmecophyte and it is defended by ants (Tillberg, 2004; R. Dirzo,

pers. observ.). *Cordia elaeagnoides* does not have any apparent indirect defences. The domatia of *C. alliodora* are naturally hollow swellings produced at most branch nodes (Trager & Bruna, 2006). These nodes may be inhabited by different ant species in different parts of the plant's range (Tillberg, 2004), but *Azteca* species are the most common ant partners of *C. alliodora* throughout the plant's geographical range (Longino, 1996). In the case of *C. alliodora* at Chamela, the age at which the mutualistic interaction with *Azteca* ants begins differs among individuals, but ant colonization can be readily assessed by direct inspection. The approximate minimum size at which *C. alliodora* individuals recruit defenders is 65 cm overall height (R. Dirzo, pers. observ.).

Of the two selected Euphorbiaceae, *Croton suberosus* has indirect defences whereas *Croton pseudoniveus* does not (R. Dirzo, unpubl. data). *Croton suberosus* is distributed on the lowlands of Jalisco, Michoacán, Guerrero and Oaxaca, on the Pacific coast of Mexico. The floral nectar of *C. suberosus* attracts wasps which prey on herbivores (Domínguez *et al*., 1989). Although larvae of *Hypercombe* sp. (Lepidoptera: Arctiidae) readily consume leaves of *C. suberosus* in laboratory test trials, larvae experimentally placed on leaves of *C. suberosus* plants in the field are readily killed and removed by wasps (Domínguez *et al*., 1989).

We sampled an area of forest approximately 1.5 km^2. Sampled plants were interspersed with plants from other species. For the purpose of this experiment, we used the following operational definitions of young (uncolonized) and old (colonized) individuals. In *Croton suberosus* (a shrub), we selected plants less than 30 cm tall for the young group and plants taller than 60 cm for the old group. Old *Croton suberosus* plants ranged between 0.6 and 3 m and they all had flowers. For the other three (tree) species, we selected plants less than 50 cm for the young group and plants taller than 1.5 m for the old group. Above this threshold, we estimated the herbivory of the smallest individuals we found. To avoid the risk that some very young plants of *Cordia alliodora* might have been colonized by ants, we verified that none of the young plants selected for this study had developed such an association. None of the young individuals of *Croton suberosus* had flowers at the time of sampling, whereas flowers were present on all old sampled individuals of this species.

Preference experiment

We chose caterpillars of *Hypercombe* sp. for the experimental work because this species is a generalist feeder, which feeds regularly on all four plant study species (Domínguez *et al*., 1989). We collected caterpillars from the field each morning and used them for preference trials the same day. We kept caterpillars in plastic containers, covered with mosquito netting for ventilation, with wet cotton wool for moisture and a mixture of native plants, not including the study species, to feed on. Six hours before the onset of the experiment, we replaced the plant material in the containers with lettuce (*Lactuca sativa* L.) to minimize the effect of previous diet. After 2 h, we removed the lettuce and starved caterpillars for 4 h. This procedure standardized caterpillar hunger (see Dirzo, 1980).

We determined each caterpillar's preference of two plants, each plant from a different group. We used only mature leaves (i.e. in positions 3–4 of the phylotaxis) for the trials. We cut out two 4-cm^2 pieces from the mature leaves of each plant and placed the four squares along the wall of a plastic container, equally spaced. We arranged the leaf pieces so that the two squares from the same plant were across from each other. We placed one caterpillar in the centre of the container and removed it after 5 h of feeding. At the end of each trial, we photographed the remaining leaf material beside a 5-cm scale bar and determined the area consumed by the caterpillar using IMAGE J (Rasband, 2003). This procedure was used for two experiments. In the first one, we tested whether caterpillars preferred to eat species with indirect defences to species without them; the second tested whether caterpillars preferred the defended (old) ontogenetic stage to the undefended (young) one.

To test whether caterpillars preferred to eat species with indirect defences to species without them, we presented caterpillars with leaf material from two individuals from the same genus (*Cordia* or *Croton*) and ontogenetic stage (young or old) but of different species (one with, the other without indirect defences). We calculated caterpillar preference by estimating the difference in leaf area consumed between species with and without indirect defences. We replicated the trials 15 times for each species pair to assess interspecific differences in direct defences. Preference for the species with indirect defences was analysed with a full factorial ANOVA with genus and ontogenetic stage as fixed factors. Because the variance of the residuals differed between groups, we rank transformed our dependent variable to fulfil the assumptions of the ANOVA model. This transformation is correct and powerful for 2×2 factorial designs like the one used for this experiment (Thomson, 1991).

To test whether caterpillars preferred the defended (old) ontogenetic stage to the undefended (young) one, we presented caterpillars with leaf material from two individuals belonging to the same species, but in different ontogenetic stages (one young individual,

one old individual). We calculated the preference of caterpillars for old individuals as the area of leaf material consumed of the old individual minus the amount consumed of the young individual. We conducted 15 replicates for each choice test comparing young and old plants within each of the four species. Preference for old individuals was analysed with a full factorial ANOVA, with genus and presence of indirect defences as fixed factors.

Field observations

To test if caterpillar preference explains herbivory in natural conditions, we conducted field observations of herbivory. We measured herbivory level and plant size (height, trunk diameter and number of leaves) of 15 individuals of each study species. In order to estimate the degree of herbivory in individual plants, we visually categorized damage using the method described in Dirzo & Domínguez (1995). We first determined a level of damage as a percentage for each leaf and then assigned leaves to different categories 0, 0–20, 20–40, 40–60, 60–80, 80–100 and 100. These seven categories were given a label of $i = 0$ to $i = 6$ and the expected percentage of leaf consumed (c_i) for each category was the midpoint of its range. We denoted the number of leaves in category i as n_i, and the number of leaves sampled as N. With these conventions, the percentage of leaf material consumed for an entire plant can be approximated by $H = \Sigma[(n_i\ c_i)]/N$. We calculated H on the basis of all the leaves in a plant except for large trees, where we measured the herbivory of the leaves on three branches chosen at random.

We tested for effects of ontogenetic class (young or old), genus (*Cordia* or *Croton*) and indirect defences (presence or absence) and their interactions on our measure of herbivory with a full factorial ANOVA. Initially, we analysed the data including height, trunk diameter and number of leaves as covariates, but we removed them from the analysis because they did not have any significant effect on our measure of herbivory. Because the residuals of the model were not normally distributed, we used Monte Carlo simulations to calculate empirical *P*-values for this analysis (Davison & Hinkley, 2006). We ran a total of 1999 Monte Carlo simulations using the statistical package R, version 2.8.1 (R Development Core Team, 2008).

RESULTS

Preference experiment

Caterpillars showed a clear preference for species with indirect defences. Regardless of the genus and ontogenetic stage, caterpillars consumed 4.98 ± 2.39 cm^2 (mean ± SE) more leaf material from species with indirect, biotic defences than from species without them ($F_{1,56} = 267.07$; $P < 0.01$; Fig. 1). There was a significant effect of the age class–genus interaction on the preference for species with indirect defences ($F_{1,56} = 7.17$, $P < 0.01$): the preference for young *Cordia alliodora* over young *C. elaegnoides* was smaller than for the other groups (Fig. 1).

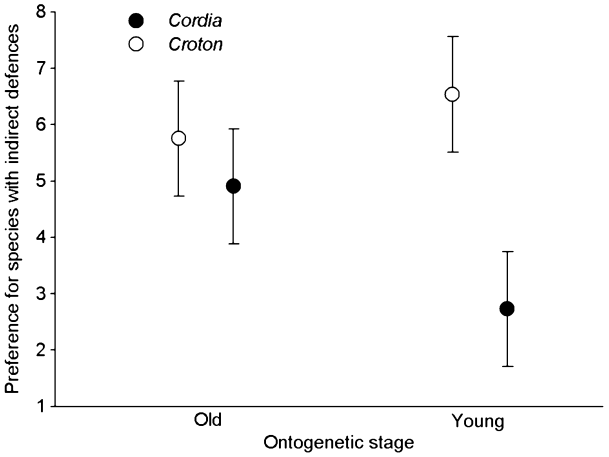

Figure 1. Preference (mean ± 95% CI) for species with indirect defences in the comparison with vs. without indirect defences for the two genera (*Croton* and *Cordia*) and the two ontogenetic stages (adult and young) of this study.

Overall, there was a marginally significant effect of within-species ontogenetic stage on the amount of leaf material the caterpillars consumed ($F_{1,56} = 4.28$; $P = 0.04$), but there was a significant interaction between ontogenetic stage and plant defensive strategy ($F_{1,56} = 15.58$, $P < 0.01$). Caterpillars preferred leaves from older individuals of plant species without them (Fig. 2). Neither genus ($F_{1,56} = 0.32$, $P = 0.57$) nor its interaction with presence of indirect defences ($F_{1,56} = 0.69$, $P = 0.41$) had an effect on the preference for old individuals.

Field observations

Only the presence or absence of indirect defences had a statistically significant effect on natural levels of herbivory (Table 1): species with indirect defences experienced less herbivory in the field than species without indirect defences (Fig. 3, $P = 0.03$). None of the other factors, or their interactions, had significant effects, with the exception of the interaction between presence or absence of indirect defences and ontogenetic stage that was also significant ($P = 0.04$). Although, overall, plants with indirect defences suffered less herbivory than plants without indirect defences, the difference was marked mainly in old plants and it was much less so in young plants (Fig. 3).

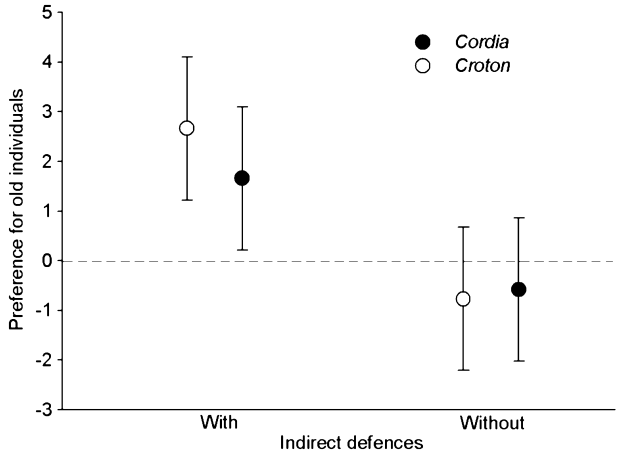

Figure 2. Preference (mean ± 95% CI) for the adult stage in the comparison adult vs. young stage for species with and without indirect defences and for the two genera (*Croton* and *Cordia*). The dashed line indicates indifference between the two ontogenetic stages. Positive values indicate a preference for old individuals and negative values a preference for young individuals.

Table 1. Results of ANOVA testing the effect of ontogenetic stage (colonized vs. uncolonized), genus (Cordia vs. Croton) and indirect defences (present vs. absent) on the herbivory index

Source of variation	d.f.	*F*	*P*
Genus	1	0.003	0.956
Indirect defences	1	4.840	0.026
Stage class	1	0.044	0.830
Genus × indirect defences	1	0.532	0.478
Genus × stage class	1	0.887	0.363
Indirect defences × stage class	1	3.851	0.038
Genus × indirect defences × stage class	1	1.398	0.243
Error	112		

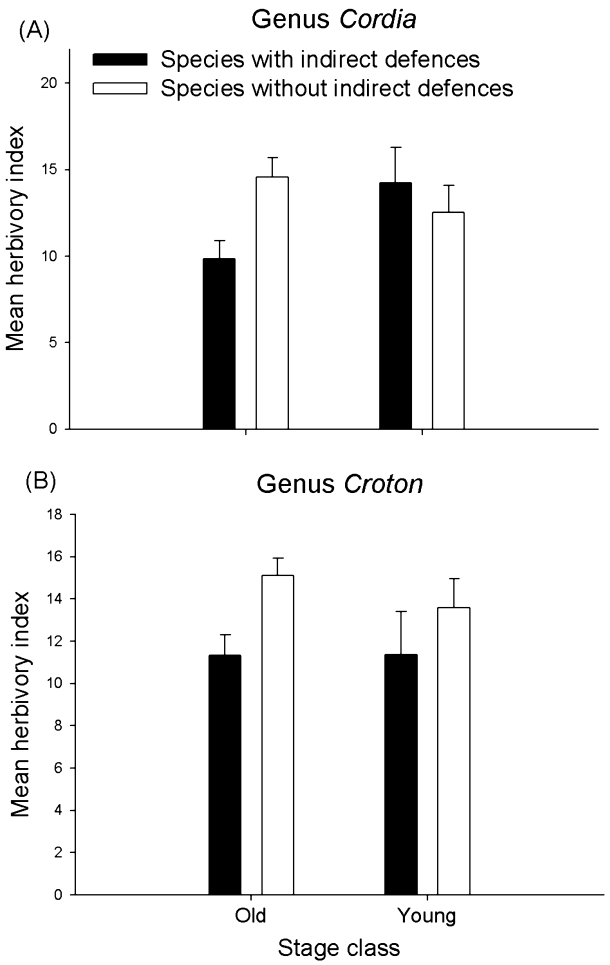

Figure 3. Mean herbivory indices of species with and without indirect defences divided by stage classes within the genera (A) *Cordia* and (B) *Croton*. Bars denote standard errors.

DISCUSSION

Our results are consistent with the assumption that defensive traits are costly. Because plants have a finite amount of resources, they face a trade-off between investing in different defensive strategies (Janzen, 1966; Heil *et al*., 1999, 2000; Nomura, Itioka & Itino, 2000; Eck *et al*., 2001). This is not a new idea, but it remains controversial. Agrawal (2006) pointed out two major difficulties when comparing defensive strategies between species with and without indirect defences. First, some studies have failed to take into account phylogenetic relationships when comparing between species with and without indirect defences. Second, many studies have concentrated on only searching for univariate trade-offs. For instance, Heil *et al*. (2000) compared chitinase activity between leaves of a myrmecophytic species and three other non-myrmecophytic species (see also Rehr *et al*., 1973; Eck *et al*., 2001). These studies implicitly assumed that the focal chemical (or the family of chemicals) constitutes a defence in itself. However, plants relying on direct defences to discourage herbivore attacks mount complex defensive systems combining different defensive traits. Even if there is a trade-off between investing in indirect or direct defences, there is no reason to expect a negative correlation between investment in indirect defences and any one particular component of the direct defence strategy.

In one of the most comprehensive studies on trade-offs between investing in indirect and direct defences, Heil *et al*. (2002) found no clear evidence that plants with indirect defences invest less in chemical defences. However, their results were inconclusive

because of the use of an artificial diet, which lacked physical defences, was rich in proteins and had concentrations of chemical defences much lower than those found in the actual leaves. This is critical because the effect of toxin concentration in the diet on consumption and growth is non-linear (Yu, 1987; Wadleigh & Yu, 1988; Johnson, 1999) and depends on the protein-to-carbohydrate ratio of the food (Simpson & Raubenheimer, 2001).

The results of our within-species comparisons (Fig. 2) are consistent with the requirement for plants to trade-off between investing in indirect and direct defences. Plants lacking indirect defences did not show a significant difference in their palatability as they matured, but for species with indirect defences caterpillars preferred plants that had already acquired the indirect defences. That individuals lose or reduce their direct defences during the adult stages is an indication that these defences are costly. This pattern fits the prediction of Boege & Marquis (2005), which states that there should be an increase in defence from the juvenile to the mature stage, and it highlights the importance of mechanistic constraints influencing ontogenetic variation in plant defences (Barton & Koricheva, 2010).

As in the present study, some previous studies have found that, in plant species with indirect defences as adults, investment in direct defences decreases when indirect defences become available (Nomura *et al*., 2001). This is not, however, a universal result. In some cases, the inconsistency in results can be explained on the basis of methodological differences, such as differences in the criteria defining the ontogenetic stages or use of specialist herbivores (Trager & Bruna, 2006), but inconsistencies between experimental results cannot always be explained purely on methodological grounds (e.g. Del Val & Dirzo, 2003). It is therefore important to note that there is no single general pattern in the ontogeny of plant defence (Barton & Koricheva, 2010) and that there is as yet no theory that adequately explains the variety of changes in plant defensive strategy deployed through ontogeny (Boege & Marquis, 2005; Barton & Koricheva, 2010).

Despite the caterpillar's preference for species with indirect defences, standing herbivory in the field was higher in plants without indirect defences than with indirect defences. This pattern was particularly clear among old plants (Fig. 3). There are two likely explanations for this pattern. Firstly, the low herbivory level of potentially preferred plants (those with indirect defences) could be a result of generalist herbivores being forced to feed on their unpreferred food sources because of the high predation risk (in *Croton suberosus*) or caterpillar attack and subsequent falling to the ground (in *Cordia alliodora*) in their preferred food sources (Lima & Dill, 1990). Secondly, the direct defences of plant species without indirect defences may repel generalist herbivores in the field, and the high herbivory we observed could attest to the presence of specialised herbivores well equipped to deal with direct plant defences. Either way, plants with indirect defences proved to be better protected against herbivores than plants without them (see also Dyer *et al*., 2004).

Finally, we suggest that trade-offs between alternative defensive traits must be one of the basic assumptions in any attempt to explain the evolution of defence strategies. That these trade-offs exist is testament to the fact that not all plant species rely on indirect defences to remove herbivores. If, in accordance with the results of our field observations, plants with indirect defences suffer less herbivory than plants without them, why, then, do many plant species not use indirect defences? We posit two possible explanations. First, that phylogenetic inertia prevents some plant species from evolving indirect defences or, second, that indirect defences are costly, so that the cost of recruiting herbivore predators balances the benefit obtained from them. Given the broad taxonomic distribution of indirect defences, it seems unlikely that phylogenetic constraints prevent other plants from adopting this defence strategy. It seems reasonable to suggest that the presence or absence of indirect defences can be explained with a cost-benefit framework. Clearly, this is an aspect that warrants further research. We conclude that indirect defences are an effective defence against herbivores and that their recruitment allows plants to reduce investment in other defence mechanisms, supporting the notion of plants trading-off between defensive strategies.

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REFERENCES

Agrawal AA. 2006. Macroevolution of plant defense strategies. *Trends in Ecology and Evolution* **22:** 103–109.

Barton KE, Koricheva J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *American Naturalist* **175:** 481–493.

Boege K. 2004. Induced responses in three tropical dry forest plants species – direct and indirect effects on herbivory. *Oikos* **107:** 541–548.

Boege K, Marquis RJ. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* **20:** 441–448.

Bryant JP, Reichardt PB, Clausen TP, Provenza FD, Kuropat PJ. 1992. Woody plant–mammal interactions. In: Rosenthal GA, Berenbaum MR, eds. *Herbivores: their interactions with plant secondary metabolites*. San Diego, CA: Academic Press, 344–371.

Bullock SH. 1986. Climate of Chamela, Jalisco, and trends in the south coastal region of Mexico. *Archives for Meteorology, Geophysics and Bioclimatology Series B* **36:** 297–316.

Chamberlain SA, Holland JN. 2009. Quantitative synthesis of context dependency in ant–plant protection mutualisms. *Ecology* **90:** 2384–2392.

Collantes HG, Gianoli E, Niemeyer HM. 1997. Effect of defoliation on the patterns of allocation of a hydroxamic acid in rye (*Secale cereale*). *Environmental and Experimental Botany* **38:** 231–235.

Cuautle M, Rico-Gray V. 2003. The effect of wasps and ants on the reproductive success of extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Functional Ecology* **17:** 417–423.

Davison AC, Hinkley D. 2006. *Bootstrap methods and their applications*, 8th edn. Cambridge: Cambridge Series in Statistical and Probabilistic Mathematics.

Del Val E, Dirzo R. 2003. Does ontogeny cause changes in the defensive strategies of the myrmecophyte *Cecropia peltata*? *Plant Ecology* **169:** 35–41.

Del Val E, Dirzo R. 2004. Myrmecophily: plants with their own army. *Interciencia* **29:** 673–677.

Di Giusto B, Anstett MC, Dounias E, McKey DB. 2001. Variation in the effectiveness of biotic defence: the case of an opportunistic ant–plant protection mutualism. *Oecologia* **129:** 367–375.

Dirzo R. 1980. Experimental studies on slug–plant interactions. I. The acceptability of thirty plant species to the slug *Agriolimax caruanae*. *Journal of Ecology* **68:** 981–998.

Dirzo R, Domínguez CA. 1995. Plant–herbivore interactions in Mesoamerican tropical dry forests. In: Bullock SH, Medina E, Mooney HA, eds. *Seasonally dry tropical forests*. Cambridge: Cambridge University Press, 304–325.

Domínguez CA, Dirzo R, Bullock SH. 1989. On the function of floral nectar in *Croton suberosus* (Euphorbiaceae). *Oikos* **56:** 109–114.

Dyer LA, Letourneau DK, Dodson CD, Tobler MA, Stireman JO, Hsu A. 2004. Ecological causes and consequences of variation in defensive chemistry of a Neotropical shrub. *Ecology* **85:** 2795–2803.

Eck G, Fiala B, Linsenmair KE, Hashim RB, Proksch P. 2001. Trade off between chemical and biotic anti-herbivore defense in the Southeast Asian plant genus *Macaranga*. *Journal of Chemical Ecology* **27:** 1979–1996.

Fiala B, Grunsky H, Maschwitz U, Linsenmair KE. 1994. Diversity of ant–plant interactions – protective efficacy in *Macaranga* species with different degrees of ant association. *Oecologia* **97:** 186–192.

Fiala B, Maschwitz U. 1992. Food bodies and their significance for obligate ant-association in the tree genus *Macaranga* (Euphorbiaceae). *Botanical Journal of the Linnean Society* **110:** 61–75.

Gaume L, McKey D, Terrin S. 1998. Ant–plant–homopteran mutualism: how the third partner affects the interaction between a plant-specialist ant and its myrmecophyte host. *Proceedings of the Royal Entomological Society of London. Series B* **265:** 569–575.

Goralka RJL, Langenheim JH. 1996. Implications of foliar monoterpenoid variation among ontogenetic stages of California bay tree (*Umbellularia californica*) for deer herbivory. *Biochemical Systematics and Ecology* **24:** 13–23.

Goralka RJL, Schumaker MA, Langenheim JH. 1996. Variation in chemical and physical properties during leaf development in California bay tree (*Umbellularia californica*): predictors regarding palatability for deer. *Biochemical Systematics and Ecology* **24:** 93–103.

Gowda JH, Albrectsen BR, Ball JP, Sjöberg M, Palo RT. 2003. Spines as a mechanical defence: the effects of fertiliser treatment on juvenile *Acacia tortilis* plants. *International Journal of Ecology* **24:** 1–4.

Heil M, Delsinne T, Hilpert A, Schürkens S, Andary C, Linsenmair KE, Sousa SM, McKey D. 2002. Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos* **99:** 457–468.

Heil M, Fiala B, Boller T, Linsenmair KE. 1999. Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften* **86:** 146–149.

Heil M, McKey D. 2003. Protective ant–plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics* **34:** 425–453.

Heil M, Staehelin C, McKey D. 2000. Low chitinase activity in *Acacia* myrmecophytes: a potential trade-off between biotic and chemical defences? *Naturwissenschaften* **87:** 555–558.

Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67:** 283–335.

Janzen DH. 1966. Coevolution of mutualism between ants and Acacias in Central America. *Evolution* **20:** 249–275.

Janzen DH. 1969. Allelopathy by mirmecophytes: the ant *Azteca* as an allelopathic agent of *Cecropia*. *Ecology* **50:** 147–153.

Johnson KS. 1999. Comparative detoxification of plant (*Magnolia virginiana*) allelochemicals by generalist and specialist Saturniid silkmoths. *Journal of Chemical Ecology* **25:** 253–269.

Kost C, Heil M. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology* **6:** 237–248.

Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68:** 619–640.

Longino JT. 1996. Taxonomic characterization of some live-stem inhabiting *Azteca* (Hymenoptera: Formicidae) in Costa Rica, with special reference to the ants of *Cordia* (Boraginaceae) and *Triplaris* (Polygonaceae). *Journal of Hymenoptera Research* **5:** 131–156.

Madden D, Young TP. 1992. Symbiotic ants as an alternative defense against giraffe herbivory in spinescent *Acacia drepanolobium*. *Oecologia* **91:** 235–238.

Nomura M, Itioka T, Itino T. 2000. Variations in abiotic defense within myrmecophytic and non-myrmecophytic species of *Macaranga* in a Bornean dipterocarp forest. *Ecological Research* **15:** 1–11.

Nomura M, Itioka T, Murase K. 2001. Non-ant antiherbivore defenses before plant–ant colonization in *Macaranga myrmecophytes*. *Population Ecology* **43:** 207–212.

R Development Core Team. 2008. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rasband W. 2003. Image J. 1.30. National Institutes of Health. Available at http://rsb.info.nih.gov/ij/

Rehr SS, Feeny PP, Janzen DH. 1973. Chemical defence in Central American non-ant Acacias. *Journal of Animal Ecology* **42:** 405–416.

Rocha CFD, Bergallo HG. 1992. Bigger ant colonies reduce herbivory and herbivore residence time on leaves of an ant-plant *Azteca muelleri* vs *Coelomera ruficornis* on *Cecropia pachystachya*. *Oecologia* **91:** 249–252.

Rohde S, Molis M, Wahl M. 2004. Regulation of anti-herbivore defence by *Fucus vesiculosus* in response to various cues. *Journal of Ecology* **92:** 1011–1018.

Rosumek FB, Silveira FAO, Neves FS, Barbosa NPU, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* **160:** 537–549.

Rudgers JA, Strauss SY, Wendel JF. 2004. Trade-offs among anti-herbivore resistance traits: insights from Gossypieae (Malvaceae). *American Journal of Botany* **91:** 871–880.

Schemske DW. 1980. The evolutionary significance of extrafloral nectar production by *Costus woodsonii* (Zingiberaceae) – an experimental analysis of ant protection. *Journal of Ecology* **68:** 959–967.

Schupp EW. 1986. *Azteca* protection of *Cecropia*: ant occupation benefits juvenile trees. *Oecologia* **70:** 379–385.

Simpson SJ, Raubenheimer D. 2001. The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. *Ecology* **82:** 422–439.

Steward JL, Keeler KH. 1988. Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae)? *Oikos* **53:** 79–68.

Stoner KE. 2005. Phyllostomid bat community structure and abundance in two contrasting tropical dry forests. *Biotropica* **37:** 591–599.

Thomson GL. 1991. A note on the rank transform for interactions. *Biometrika* **78:** 697–701.

Tillberg CV. 2004. Friend or foe? A behavioral and stable isotopic investigation of an ant–plant symbiosis. *Oecologia* **140:** 506–515.

Trager MD, Bruna EM. 2006. Effects of plant age, experimental nutrient addition and ant occupancy on herbivory in a neotropical myrmecophyte. *Journal of Ecology* **94:** 1156–1163.

Wadleigh RW, Yu SJ. 1988. Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. *Journal of Chemical Ecology* **14:** 1279–1284.

Webber BL, Moog J, Curtis ASO, Woodrow IE. 2007. The diversity of ant–plant interactions in the rainforest understorey tree, *Ryparosa* (Achariaceae): food bodies, domatia, prostomata and hemipteran trophobionts. *Botanical Journal of the Linnean Society* **254:** 353–371.

Yu SJ. 1987. Microsomal oxidation of allelochemicals in generalist (*Spodoptera frugiperda*) and semispecialist (*Anticarsia gemmatalis*). *Journal of Chemical Ecology* **13:** 423–436.